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MODERATE REGULAR EXERCISES REDUCE INFLAMMATORY RESPONSE FOR PHYSICAL STRESS

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Abstract : Exercises induce pro-inflammatory cytokines. We assessed the effect of different grades of exercises on inflammatory cytokine response. Twenty healthy volunteers performed a single bout of moderate exercise, a single bout of strenuous exercise and one month regular moderate exercise using standardized 10m Shuttle Walk Test. Interleukin-6 (IL-6) and Tumour Necrosis Factor Alpha (TNF-∝) were estimated by Sandwich ELISA method after each exercise regime. Statistics were run using SPSS software version 11.0, Systat software. Repeated measures ANOVA has been used for analysis of IL-6 values and Friedman test has been used for analyzing TNF-∝ and IL-6 values. Twenty healthy volunteers (18 to 30 years) were chosen for this study. The mean and SEM of plasma levels (pg/ml) of IL-6 before exercise was 10.70±1.11 pg/ml, whereas, after acute moderate exercise and acute strenuous exercise it was 12.00±1.09 pg/ml and 13.35±0.89 pg/ml respectively. Interestingly, after one month of moderate exercise the values decreased to; 8.80±0.65 pg/ml. Mean and SEM of TNF-α before exercise was 121.78±29.06 pg/ml. With acute moderate exercise and after acute strenuous exercise the values were 132.90±35.75 pg/ml and 112.05±29.89 pg/ml respectively. After one month moderate exercise the levels decreased to 94.95±27.29 pg/ml. The observed changes in both IL-6 and TNF-α levels before and after both moderate and strenuous exercise were statistically significant. Although there was a slight decrease in the value of both the cytokines after one month of regular moderate exercise compared to baseline value, the difference in the values was not statistically significant. However, both IL-6 and TNF-∝ levels showed overall statistically significant difference among the different grades of exercise. Plasma IL-6 and TNF-∝ increase with acute moderate exercise and IL-6 increases further with acute strenuous exercise. Their levels tend to fall below baseline with one month of regular moderate exercise indicating that regular moderate exercise has beneficial effects.

Key words : interleukin-6 tumour necrosis factor alpha exercise

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INTRODUCTION

Increased levels of TNF-α and IL-6 have often been demonstrated to be one of the predictors of increased risk for development of lifestyle diseases such as hypertension, coronary artery disease and diabetes mellitus. Exercises of moderate nature have been shown to reduce the incidence of cardiovascular diseases (1). Regular exercise improves the health and stabilizes the immune system (1-6). Many diseases have been recently attributed to persisting inflammation. During the last two decades, several studies have been carried out to monitor the changes in cytokine levels with different grades of exercises. There have been studies on subjects undertaking marathon running, military training, downhill running on a treadmill, cycling, etc., on different groups of individuals in different parts of the world (7-10). The observations of these studies are not uniform. Most of them indicate rise in the levels of inflammatory cytokines following burst of exercise of varying levels. The reports of relationships between the cytokines IL-6 and TNF-α are variable (11, 12). There are studies which indicate that high end athletic activities do increase infections as well as cardiovascular mortality (13-15). The issue that still remains to be addressed is what level of exercise will help in the overall improvement of the general health. Which of these responses is the best for maintenance of a good immune status ?

The impact of exercise on various cytokines has been described differently in different studies. These variations have been attributed to different exercise regimen, pre-existing competition-stress, as well different patterns of exercises and different applications of statistics and methods. We undertook this study to understand the influence of a single bout of moderate exercise and a single bout of strenuous exercise on the plasma levels of TNF-α and IL-6 and compare them with the levels at the end of one month of moderate exercise in healthy individuals leading a sedentary life.

METHODS

Subjects

20 healthy volunteers, not performing any kind of regular exercise were included in the study after obtaining their consent. Institutional ethics committee of M.S. Ramaiah Medical College, Bangalore, approved the study. The subjects were excluded if they had any allergic disease, chronic viral infections like HIV, Hepatitis B and C, chronic metabolic diseases like diabetes and hypothyroidism. Individuals with hypertension were also excluded

Exercise regimen

All subjects were made to perform a single bout of moderate exercise (acute moderate exercise), a single bout of strenuous exercise (acute strenuous exercise) and a scheduled moderate exercise regime everyday for one month. The subjects were made to perform acute moderate exercise on the first day and acute strenuous exercise on the second day. They were made to perform scheduled regular moderate exercise from third day onwards, for 30 days with strict monitoring. The exercise was graded as moderate or strenuous based on the rise in heart rate. It was labelled as moderate

when the heart rate increased by 50% from resting level and was labelled as strenuous when heart rate doubled (16-18). Heart rate was also used for grading exercise on a daily basis as measuring the heart rate is simple, in-expensive and reliable. Heart rate was estimated by using the standard Heart Rate Monitor Machine (HRM) from Polar Company (RS-100). It was worn on the wrist of the subjects like a watch as specified in the manual.

Shuttle walk test protocol

The exercise regime chosen was the standardized 10 m Shuttle Walking test regime, described by Glenfield Hospital, Leicester, United Kingdom in collaboration with the department of Physical Education and Sports Science, Loughborough University of Technology, United Kingdom (19-22). In this exercise protocol, the subjects walk on a 10 m plain path at the 2 ends of which are placed marker cones. The subjects walk between the cones corresponding to the beeps given out by a record player. Subjects have to increase their speed of walking gradually according to the shortening of intervals between the consecutive beeps as time progresses. The aim of the study was to look into the effect of a moderate exercise.

Analysis of samples

A venous blood sample from cubital vein (using vacutainers) was collected just before acute moderate exercise (baseline). Another sample was collected immediately after acute moderate exercise on the same day. After performance of acute strenuous exercise on the next day, the sample was collected again. Blood sample was also collected from individuals after one month of scheduled regular moderate exercise on the last day after the exercise. All the samples were obtained between 1 pm and 2 pm. The samples collected from each individual were aliquoted and stored at –40°C till further analysis. The plasma sample was used to estimate the levels of cytokines IL-6 and TNF-∝, by using the ELISA method. ELISA was performed using DuoSet ELISA development system as per the manufacturer's instructions (R & D systems, USA). Briefly, polystyrene microtiter plates (NUNC, U16 Maxisorp type, Denmark) were coated with monoclonal capture antibody (antihuman TNF-alpha antihuman IL-6) obtained from mouse (R & D systems, USA) and incubated at 4°C overnight. The following day, the plates were blocked and then incubated for 2 hours with plasma. This was followed by addition of corresponding biotinylated detection antibody obtained from goat (R & D systems, USA) and incubated for 2 hours. Subsequently strepatavidin horseradish peroxidase (R & D systems, USA) and tetra methyl benzidine substrate (Bangalore Genie, India) were added. The reaction was stopped using 2 N sulphuric acid and optical density (O.D) readings were recorded 450 nm (ELISA reader: Organon Teknika Microwell system, Reader 230s, Germany). All the experiments were conducted in duplicates. A standard curve was obtained based on the standards provided by the manufacturer. The results were expressed as concentration of cytokines (in pg/ml) read from the standard curve.

RESULTS

Twenty healthy volunteers in the age group 18 to 30 (Mean and SD: 21.05±2.37)

were selected for the study. There were 11 males and 9 females. The demographic details of the subjects are as follows: For males; height: 1.68±0.07m, weight: 55±3.2 kgs, BMI: 20.2±1.2. For females: height: 1.58±0.06m, weight: 55.4±3.5 kgs, BMI: 22.43±1.5. IL-6 and TNF-∝ levels were measured before and after different grades of exercises. There was a significant rise in the levels of both IL-6 and TNF-∝ with both acute moderate and acute strenuous exercise when compared with the respective levels before the exercise (baseline value). There was a slight decrease in the levels of these two cytokines after one month of regular moderate exercise which was not significant (Table – Ia and IIa).

Percentage of subjects whose IL-6 levels increased from baseline is 60.0% after Moderate exercise and 60.0% after strenuous exercise, while this percentage was 20.0% after one month of moderate exercise which is statistically significant at p=0.019. Similarly, the percentage of subjects whose IL-6 levels decreased from baseline was 35.0% after moderate exercise and 35.0% after strenuous exercise, while it was 70.0% after one month moderate exercise at p=0.060, which may be considered close to statistical significance. (Ref: Table – Ib). Percentage of subjects whose TNF-a levels increased from baseline was 63.2% after moderate exercise and was 52.6% after strenuous exercise, while it was 15.8% after one month moderate exercise with p=0.007 which is statistically significant. Further, Percentage of subjects whose TNF-a levels decreased from baseline was 36.8% after moderate exercise and 47.4% after strenuous exercise, while it was 73.7% after one month

TABLE Ia : Effect of graded Exercise on IL6.

IL-6 levels Concentration in pg/ml (n=20)	*Range*	*Mean±SEM*
No Exercise	4.00–20.0	10.70±1.11[a]
Moderate Exercise	4.00–22.0	12.00±1.09[b]
Strenuous Exercise	5.00–20.0	13.35±0.89[bc]
One month moderate Exercise	4.00–13.0	8.80±0.65[d]
Significance	ANOVA Repeated measures, F=6.305 p=0.001	

Non-identical superscripts are significant at 5% level of significant by paired student t test while the identical superscripts are non-significant.

TABLE Ib : Number and percentage of subjects having improved, decreased and remained same in IL-6 after receiving graded level of exercise (n=20).

Changes in IL6	*After moderate exercise*	*After strenuous exercise*	*After one month moderate exercise*
Number of subjects increased in IL6 from baseline :	12 (60.0%)	12 (60.0%)	4 (20.0%)
Number of subjects decreased in IL6 from baseline :	7 (35.0%)	7 (35.0%)	14 (70.0%)
Number of subjects remained same from baseline :	1 (5.0%)	1 (5.0%)	2 (10.0%)

TABLE IIa : Effect of graded exercise on TNF-α levels.

TNF-α levels (pg/ml)	*Range*	*Mean±SEM*
No-Exercise	5.00–425.00	121.78±29.06[a]
Moderate exercise	4.00–620.00	132.90±35.75[b]
Strenuous exercise	4.00–520.00	112.05±29.89[abc]
One month moderate exercise	5.00–425.00	94.95±27.29[ad]
Significance[#]	Repeated Measures ANOVA F=7.011 p=0.006**	

Non-identical superscripts are significant at 5% level of significant by paired student t test while the identical superscripts are non-significant. Significance tests were carried out after natural log transformation.

TABLE IIb : Number and percentage of subjects having improved, decreased and remained same in TNF-α after receiving graded level of exercise (n=19*).

Changes in IL6	*After moderate exercise*	*After strenuous exercise*	*After one month moderate exercise*
Number of subjects increased in TNF-α from baseline :	12 (63.2%)	10 (52.6%)	3 (15.8%)
Number of subjects decreased in TNF-α from baseline :	7 (36.8%)	9 (47.4%)	14 (73.7%)
Number of subjects remained same from baseline :	–	–	2 (10.5%)

*One subject value of parameter is not obtained.

moderate exercise with p=0.060 which is also appears to be close to statistical significance (Table IIb). Significance test by paired proportion test was estimated using the significance test available online, developed by Prof Hossien Ashram, University of Baltimore, USA.

DISCUSSION

We observed that in both moderate and strenuous exercises, there were significant elevations in both IL-6 and TNF-α level, in unaccustomed normal subjects (Table – Ia and Table – IIa). The elevation of IL-6 was in equal percentage of subjects in response to moderate and strenuous exercise, while the number of subjects with increase in TNF-α decreased from 12 to 10 when they performed strenuous exercise. On performing continued moderate exercises for a month, the number of subjects whose levels of TNF-α as well as IL-6 decreased significantly compared to the initial baseline value (Table – Ib and Table – IIb).

The finding of triggered inflammatory cytokine production concurs with many previous publications (1-6). The exercise being used here is of moderate to severe grade and not of exhaustive nature as in our previous publication. This explains the elevated levels of TNF-α in the majority of subjects in the present study compared to the drop in the majority of non-athletes as published earlier (30). Even in the current study, there was a drop in 10% of subjects who had an elevation in TNF-α on strenuous exercise.

Gradual drop in inflammatory cytokines as well as in inflammatory markers on continued exercise practice has been demonstrated in few of the published studies especially in patients with coronary artery disease (24). This study shows a fall in IL-6 and TNF-∝ levels with regular moderate exercise, suggesting that such a decrease in the levels of these two cytokines could also be beneficial for health and immunity. It is of interest to draw the attention to our previous publication on the direct correlation of serum glucose and TNF-a levels (25). IL-6 and TNF-∝ are pro inflammatory cytokines, therefore then: overproduction leads to inflammation and tissue damage (26). Thus regular moderate exercise seems to optimise their release and thus may bring down deleterious effects of IL-6 and TNF-∝ by keeping them at levels necessary for human body with a buffer to elevate the level during sudden bursts of exercises. However, such a down regulation could also lead to increased susceptibility to some of the infections. This needs further clarification. But studies which have looked into cross sectional comparison of immunological status of athletes and non-athletes have not shown significant difference

in the susceptibility to infections (31). In a majority of the subjects, acute unaccustomed activity of moderate to mild nature was shown to elevate the pro-inflammatory cytokines. (7, 9, 25). However, this effect was not observed when healthy individuals were subjected to a strenuous exercise (30). Similarly, in the present study also (Table Ia and Table IIa), the level of TNF-α was decreased at the end of strenuous exercise and the level of IL-6 was found to decrease. The IL-6 under normal physiological circumstances inhibits TNF-∝ production, though initially both the cytokines are released from a similar source. This relationship of early drop in TNF-∝ with sustained rise in IL-6 appears to be a normal stress response for maintenance of homeostasis which is seen even in response to LPS (lipopolysaccharide) and probably reflects a physiological relationship. The effect of elevated inflammatory cytokine response seems to be attenuated in the majority of subjects, especially when they undergo regular training, but not totally ablated. Our previous studies have indicated attenuation of response but not total ablation of inflammatory response, if such individuals were to get involved in bouts of unaccustomed physical activity. This may be beneficial for sports persons and athletes who need to get involved in such sudden bouts of activity during competitions. Thus such people tolerate physical stress better. Mental stress is also known to increase plasma levels of cytokines IL-6 and TNF-∝ (27). In these individuals, the levels of these cytokines may not rise as much as it would increase in those not performing regular exercise, facing the same level of mental stress (28). Several studies have shown that in patients with atherosclerosis, Coronary Artery Disease and Diabetes Mellitus have elevated levels of IL-6 and TNF-∝ (27). Certain autoimmune disorders like Rheumatoid Arthritis are associated with increased plasma levels of pro inflammatory cytokines like IL-6 and TNF-∝, which increase joint inflammation (29). Regular moderate exercise may benefit such patients by bringing down the levels of such cytokines. What is surprising is the deviation or failure of the process that is observed in a small percentage of subjects. The reasons for such a failure need further evaluation. The level of moderate exercise to be prescribed to different individuals to achieve a stable immunological homeostasis needs to be worked out and we need to further investigate and ascertain how and by what mechanisms the levels of TNF-∝ and IL-6 alter in response to various degrees of exercises. The normal range of plasma levels of IL-6 and TNF-α are very wide. The levels for IL-6 and TNF alfa estimated in the current study are different from other populations in other studies as basal levels have not been established yet for the Indian population and hence it is not surprising that the values are different from the range suggested. Moreover, the levels of the pro-inflammatory cytokines are also altered due to psychological factors, like the amount of psychological stress the person undergoes just before starting an exercise. In this study, the volunteers are drawn from medical profession, undergraduates and postgraduate students, who are generally not used to physical exercise or physical stress. The cytokine levels obtained may also vary to an extent depending on the kits obtained from different companies. Variability is one of the major drawbacks in cytokine research. Some studies have pointed out to the fact that these cytokine levels are

highly variable (32, 33). However, since the values were measured using the same kit on the same set of individuals before and after exercises, the differences measured are reliable. The changes in the cytokine levels before and after intervention are more critical than the absolute values.

Hence performing moderate exercise on a daily basis not only has beneficial effects for health, but it also proves to be a buffer against physical stress.

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